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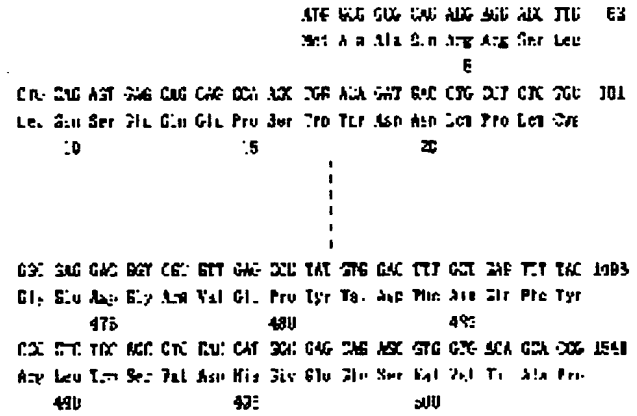
(54) TAB1 PROTEIN AND DNA CODING THE SAME

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain the subject new protein having a specific amino acid sequence, having an activity for activating TAK1 which is a factor in the signal transmission system of a transformation proliferation factor-β, and useful for searching substances stimulating or suppressing the cell proliferation, immunosuppression, etc.
SOLUTION: This new TAB1 protein has an amino acid sequence of the formula, and has an activity for activating TAK1 protein which is a factor in the signal transmission system of a transformation proliferation factor-β (TGFβ). By the utilization of a property that the new TAB1 protein combines with the TAK1, the TAB1 protein is useful for screening a substance inhibiting the combination of the TAK1 with the TAB1 and acting as an agents or antagonist against the actions of cell proliferation suppression, immunosuppression, bone differentiation, etc., The TAB1 protein is obtained by screening a genomic DNA library prepared from peripheral



leukocyte, etc., with a probe comprising a DNA coding the partial amino acid sequence of a protein obtained by the search of the protein heaving a direct interaction with the TAK1 protein.

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CLAIMS

[Claim(s)]
[Claim 1] Array number: TAB1 protein which has the amino acid sequence shown in 1.
[Claim 2] Array number: Protein which has the amino acid sequence embellished by 1 or the permutation of two or more amino acid, deletion, and/or addition to the amino acid sequence shown in 1, and has the biological property of TAB1 protein.
[Claim 3] Array number: Protein in which the code is carried out by DNA which can be hybridized to DNA which has the nucleotide sequence shown in 1 under the hybridization conditions of 60 degrees C, 0.1xSSC, and 0.1% sodium dodecyl sulfate and which has the biological property of TAB1 protein.

[Claim 4] Array number: Protein which has the amino acid sequence which consists of the amino acid of the place [21st] - the 579th place of an amino acid location in the amino acid sequence shown in 1.
[Claim 5] Array number: The polypeptide which has the amino acid sequence which consists of 68 amino acid of the amino acid locations 437-504 in the amino acid sequence shown in 1.
[Claim 6] Protein according to claim 2 whose 52nd amino acid is an arginine in the amino acid sequence shown in the array number 1.
[Claim 7] The fusion protein which grows into any 1 term of claims 1-6 including the protein or the polypeptide of a publication.
[Claim 8] The approach characterized by cultivating the host as for whom the transformation was done by the expression vector which grows into any 1 term of claims 1-7 including DNA which carries out the code of this protein or the polypeptide in the protein of a publication, or the manufacture approach of a polypeptide, and extracting this protein or a polypeptide from this culture.
[Claim 9] The approach according to claim 8 said host is a mammals cell or a yeast cell.

[Claim 10] The approach which is an approach of making any 1 term of claims 1-7 generating the protein or the polypeptide of a publication in a mammals cell, and is characterized by introducing into a mammalian cell DNA which carries out the code of this protein or the polypeptide.
[Claim 11] DNA which carries out the code of the protein or the polypeptide of a publication to any 1 term of claims 1-7.
[Claim 12] The expression vector which changes including DNA according to claim 11.
[Claim 13] The host as for whom the transformation was done by the expression vector according to claim 12.
[Claim 14] The host according to claim 13 said whose host is a mammals cell or a yeast cell.
[Claim 15] (A) or [contacting the sample which contains TGF-beta signal transfer system inhibitor in the cell which discovers TAB1 protein of a publication, or a polypeptide and TAK1 protein in any 1 term of claims 1-6] -- or the screening approach of the TGF-beta signal transfer system inhibitor characterized by what it introduces and the kinase activity of (B) TAK1 protein is measured for.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to TAB1 protein which is 1 member of the signal transfer system of transformation growth factor-beta (Transforming GrowthFactor - beta;TGFbeta), and the gene which carries out the code of it.
[0002]
[Description of the Prior Art] TGF-beta is a multifunctional factor which controls many fields of a cell function. As the whole surface, TGF-beta manages the restoration and playback of an organization accompanying various traumata. By abnormality production in TGF-beta in the trauma which became chronic, restoration of an organization and reproductive balance may collapse and morbid fibrosis may arise. Hepatic fibrosis is known as symptoms in which the balance of TGF-beta production collapsed. In liver, TGF-beta commits composition of an extra-cellular-matrix proteolytic enzyme as main causative agents of hepatic fibrosis by guiding the inhibitor of inhibition and a dialytic ferment by accelerating production of the extra-cellular-matrix protein leading to fibrosis.
[0003] As one member of the signal transfer system of the member of the super family of TGF-beta, the mitogen-activation protein kinase kinase kinase (Mitogen-Activated protein Kinase Kinase Kinase;MAPKKK) system is known.
[0004] A MAPK path is a saved eukaryon nature signal transfer system which converts the signal of an acceptor into various operations. This system contains three kinds of protein kinases, i.e., above MAPKKK, MAPKK, and MAPK. MAPK is activated by the phosphorylation by MAPKK and MAKK is activated by MAPKKK (E. — Nishida et al. —) Trends Biochem.Sci.Vol.18 and p.128 (1993); K.J.Blu mer ** — Above-shown Vol.19 and p.236 (1994); R.J.David, above-shown Vol.19, and p.470 (1994); C.J.Marchall, Cell, Vol.80, and p.179 (1995).
[0005] TAK1 which is one member of the MAPKKK family which functions in the signal transfer system of the member of a TGF-beta super family — KYamaguchi ** — it was identified (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)) . TGF-beta transmits a signal to a cytoplasm side through the HETEROMA complex of 1 mold which is the film penetration protein containing a serine - and threonine-specific kinase domain, and a 2 mold TGF-beta receptor (J. L.Wrana et al., Nature, Vol.370, p.341 (1994); D.M.Kingsley et al., Genes Dev., Vol.8, and p.133 (1994)). However, a down-stream signal transfer device is hardly known in the molecular level from a TGF-beta receptor.
[0006]
[Problem(s) to be Solved by the Invention] Therefore, this invention tends to offer the gene which carries out the code of TAB1 protein and it of the signal transfer system of a TGF-beta receptor which are one member found out newly. Furthermore, this invention offers the screening approach of TGF-beta signal transfer system inhibitor. In addition, TAB1 means the protein (TAK1Binding Protein) combined with TAK1.
[0007]
[Means for Solving the Problem] In order that this invention may solve the above-mentioned technical problem, this invention As opposed to the amino acid sequence shown in 1 array number: — TAB1 protein; which has the amino acid sequence shown in 1 — array number: — It has the

amino acid sequence embellished by 1 or the permutation of two or more amino acid, deletion, and/or addition. As opposed to DNA which has the nucleotide sequence shown in 1 And the protein; array number whose 52nd amino acid is an arginine in the amino acid sequence shown in the protein; array number 1 which has the biological property of TAB1 protein : 60 degrees C, The code is carried out by 0.1xSSC and DNA which can be hybridized under the hybridization conditions of 0.1% sodium dodecyl sulfate. And the protein; array number which has the biological activity of TAB1 protein : In the amino acid sequence shown in 1 Protein; which has the amino acid sequence which consists of the amino acid of the place [21st] - the 579th place of an amino-acid location, and an array number: Offer the polypeptide which has the amino acid sequence which consists of 68 amino acid of the place [437th] - the 504th place of an amino acid location in the amino acid sequence shown in 1.

[0008] This invention cultivates the host as for whom the transformation was done by the expression vector which changes in the above-mentioned protein or the manufacture approach of a polypeptide again including DNA which carries out the code of this protein or the polypeptide, and the approach characterized by extracting this protein or a polypeptide from this culture is offered. This invention offers the approach characterized by introducing into a mammalian cell DNA which carries out the code of this protein or the polypeptide again in the approach of making above-mentioned protein or an above-mentioned polypeptide generate in a mammals cell.

[0009] This invention offers the host as for whom the transformation was done by the expression vector which changes again including DNA and this DNA which carry out the code of aforementioned protein or an aforementioned polypeptide, and this expression vector. This invention offers the screening approach of TGF-beta signal transfer system inhibitor further.

[0010]

[Embodiment of the Invention] TAB1 protein of this invention has the property which combines with TAK1 in the signal transfer path of transformation growth factor-beta (TGF-beta), and activates TAK1. This property and many other properties are indicated by examples 2-4, and 6-10 at the detail. TAB1 protein of this invention has the amino acid sequence (array number: 1) presumed from the nucleotide sequence of cDNA by which cloning was carried out by the approach of indicating in the examples 1 and 5.

[0011] However, in the protein which has biological activity, holding the biological property in which protein by birth has what has the amino acid sequence embellished by 1 or the permutation of two or more amino acid, deletion, and/or addition is known well. Therefore, this invention contains what has the amino acid sequence embellished by 1 or the permutation of two or more amino acid, deletion, and/or addition to the amino acid sequence shown in array number:1, and has the biological property of TAB1. As the one mode, the protein whose 52nd amino acid is an arginine is mentioned in the amino acid sequence shown in the array number 1.

[0012] Furthermore, by screening a different organ from the organ of a seed or organization by which the protein was got by using the DNA as a probe, the DNA library from an organization, or the DNA library from other kinds, if cloning of the DNA which once carries out the code of the specific protein is carried out, although it has the same biological property, it is also known that DNA which carries out the code of the protein which differs in an amino acid sequence will be obtained. Therefore, this invention also includes the protein in which the code is carried out by 60 degrees C, 0.1xSSC, and DNA that can be hybridized under the hybridization conditions of 0.1% sodium dodecyl sulfate as opposed to DNA which has the nucleotide sequence shown in array number:1 and which has the biological property of TAB1 protein.

[0013] As protein with which this invention was embellished, the protein which has the amino acid sequence which consists of the amino acid of the place [21st] - the 579th place of an amino acid location in the amino acid sequence of array number:1 is mentioned, for example. This protein has the biological property of TAB1 protein. Moreover, the polypeptide which has the amino acid sequence which consists of 68 amino acid of the place [437th] - the 504th place of an amino acid location in the amino acid sequence shown in array number:1 as a polypeptide with which this invention was embellished is mentioned. By combining with TAK1, this polypeptide has the property which activates the kinase activity of TAK1. Furthermore, the protein which the above-mentioned

protein or a polypeptide has united with other protein as protein with which this invention was embellished, and has the biological activity of TAB1 is mentioned.
[0014] The protein or the polypeptide of this invention can copy the physiological function of TGF-beta by itself by activating TAK1 important for the signal transfer system of TGF-beta, and also is useful because of the screening approach of the matter which checks association of TAK1 and TAB1 using combining with TAK1, and works as the agonist to an operation of cytostatic, immunosuppression, bone differentiation, etc., or an antagonist.
[0015] DNA which carries out the code of the protein of this invention is DNA which carries out the code of the amino acid sequence shown in array number:1. Such DNA can be obtained by the approach of indicating in the examples 1 and 5, and has the nucleotide sequence of array number:1. However, an array number: DNA which carries out the code of the amino acid sequence shown in 1 does not necessarily need to have the nucleotide sequence shown in array number:1, and may consist of another codons which carry out the code of the same amino acid. For example, an array number: It can change into the thing containing the codon into which the nucleotide sequence of the Homo sapiens origin shown in 1 is efficiently translated in microorganisms, such as bacteria and yeast, and this can be performed using common knowledge techniques, such as part specification mutagenesis which uses a primer.
[0016] The array number of this invention: To the amino acid sequence shown in 1, DNA to which 1 or two or more amino acid sequences carry out the code of the protein or the polypeptide which has a permutation, deletion, and/or the amino acid sequence added can use as mold DNA which has the nucleotide sequence shown in array number:1, and can produce a part specification mutagenesis method, the PCR method, etc. using the well-known approach in itself. DNA which carries out the code of the protein or the polypeptide shortened among the protein which has the embellished amino acid sequence further again as compared with protein by birth can also be obtained by, for example, introducing a translation initiation codon and/or a translation termination codon into DNA by birth, for example, cDNA. Installation of these codons can be performed by for example, the part specification mutagenesis, the PCR method, etc. Or a suitable restriction enzyme cuts DNA by birth, for example, cDNA, and it is obtained also by adding an oligonucleotide by request.
[0017] The array number of this invention : DNA which carries out the code of the protein which can hybridize with DNA which has the nucleotide sequence shown in 1, and has the biological property of TAB1 For example, the various organizations or organ shown in an example 6, for example, the heart, a brain, a placenta, Liver, skeletal muscle, the kidney, the pancreas, a spleen, a thymus gland, a prostate gland, a testis, the ovary, a small intestine, It is carried out by screening the genomic DNA library prepared from the large intestine, the peripheral blood leucocyte, etc., or a cDNA library, using as a probe the nucleotide sequence shown in array number:1 of this invention, or its part. The above-mentioned DNA library may originate not only in the thing originating in Homo sapiens but in other animals, for example, a rat, a mouse, a rabbit, a goat, a sheep, a cow, Buta, etc.
[0018] This invention relates to the expression vector which changes including the above-mentioned DNA, and the host by whom the transformation was done by that cause again. An expression vector changes with hosts. Both a procaryote and eukaryote can be used as a host of this invention. As a procaryote, the microorganism of bacteria, for example, an ESHIERISHIA (Escherichia) group, for example, Escherichia coli, (Escherichia coli), and a bacillus (Bacillus) group microorganism (B. subtilis), for example, bacillus Subtilis etc., are used, and lower eukaryote, for example, mold, or yeast is mentioned as eukaryote.
[0019] As mold, penicillium (Penicillium) group microorganisms, such as an Aspergillus (Aspergillus) group microorganism (Aspergillus niger), for example, Aspergillus nigre, and Aspergillus oryzae (Aspergillus orizae), etc. are mentioned, and a Saccharomyces (Saccharomyces) group microorganism (Saccharomyces cerevisiae), for example, Saccharomyces cerevisiae etc., is mentioned as yeast, for example.
[0020] Moreover, as high eukaryote, an animals-and-plants cell, for example, the immortality-ized example of an animal culture tissue, for example, a COS cell, a CHO cell, NIH3 T3, etc. can be used. Moreover, the insect cell 9, for example, Sf, and Sf12 grade can also be used. The expression vector of this invention contains the manifestation control array which may function in the

above-mentioned host, for example, a promotor, besides DNA which carries out the code of the protein or the polypeptide of the invention in this application.
[0021] As a promotor of bacteria, for example, Escherichia coli, T3 and T7 grade can be used and the promotor of the gene of a glycolytic pathway enzyme, for example, GAL1 promotor, GAL4 promotor, etc. are mentioned as a promotor of yeast, for example. As a promotor for animal cells, a viral promotor, for example, a CMV promotor, an SV40 promotor, etc. are mentioned. Extraction and purification of the transformation of the host by the expression vector, culture of a host, the protein of this invention from a culture, or a polypeptide can be performed according to a conventional method. For example, from a culture, isolation and purification of protein or a polypeptide are independent, or can combine and use the stock-in-trade for isolating and refining protein or a polypeptide, for example, an ammonium-sulfate precipitation method, gel filtration technique, Opposition HPLC, etc.
[0022] This invention relates to the screening approach of TGF-beta signal transfer system inhibitor further. or [contacting the protein which has the biological activity of TAB1, and the sample which contains TGF-beta signal transfer system inhibitor in the cell which discovers TAK1 (270 K Yamaguchi et al., Sceince, vol. p 2008 (1995))] — or it introduces and, subsequently the activity of TAK1 is measured. A fusion protein with other protein is sufficient as the protein which has the biological activity of TAB1 or a polypeptide, and TAK1, and the cell which discovers these is yeast or a mammals cell. Such a screening system can be built by the approach of indicating in the examples 1, 2, 3, 4, 7, 8, and 9.
[0023] or [contacting the sample which contains TGF-beta signal transfer system inhibitor to the built screening system] — or it introduces and the kinase activity of TAK1 is measured. The measuring method of the kinase activity of TAK1 may measure the kinase activity of TAK1 self, and may measure the kinase activity of MAPKK or MAPK which exists in the lower stream of a river of TAK1 of a signal transfer system, and is activated by TAK1. Moreover, it can measure by the phenotype of the activity of the reporter gene under control of the target gene of a MAPK path, or a target gene promotor, the amount of mRNA(s), or a gene. According to the screening approach of the TGF-beta signal transfer system inhibitor of this invention, the matter which checks association of TAB1 and TAK1 which can serve as a therapy agent of the disease in which abnormality production of TGF-beta participates can be screened.
[0024]
[Example] Next, an example explains this invention still more concretely.
In order to analyze the TAK1-dependency path of functioning in example 1. TGF-beta signal transfer, it is a yeast 2-hybrid system (S. Frelds et al. and Trend Genet.10,286 (1994)). It used and looked for the protein which interacts TAK1 and directly.
[0025] First, TAK1 gene and LexA The expression vector was produced by making the gene which carries out the code of the DNA-joint domain connect. pLexA-TAK1deltaN is pBTM116 (A. B.Vojtek et al., Cell, Vol.74, and p.205 (1993)). The TAK1deltaN coding sequence (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)) which doubled and inserted the frame is contained. The code was carried out all over the Homo sapiens brain cDNA library, and in order to identify the protein which interacts with TAK1deltaN, the yeast 2-hybrid system was used.
[0026] The upstream of yeast HIS3 coding region was made to discover two hybrids in the Saccharomyces cerevisiae (Saccharomyces cerevisiae) L40 share (LYS2::LexA-HIS3) containing the unified reporter structure with which the binding site for LexA protein is arranged. If two hybrid protein interacts, transformer AKUCHIBESHON of said reporter structure can happen, and yeast can be increased under the nonexistence of a histidine (SC-His).
[0027] LexA-TAK1deltaN fusant is independent and brings about the manifestation of HIS3 of sufficient amount to make growth possible without needing the histidine from the outside. However, 40mM(s) which are the chemistry inhibitors of imidazole glycerol DEHIDOROKENAZE which is the product of HIS3 gene Histidine auxotroph can be attained by making a cell increase under existence of 3-amino trio ZORU (3-AT) (G. M.Kishore et al., Annu.Rev.Biochem.Vol.57, and p.627 (1988)).
[0028] The transformation of the yeast was carried out with the capture plasmid containing the Homo sapiens brain cDNA manifestation library clone who made this invitation (bait) plasmid connect

with the gene which carries out the code of the GAL4 activation domain (GAD). About 1x106 Electropositive clone TAB1cDNA which carries out the code of the protein was obtained from the transformant of an individual. The GAD fusion protein discovered with this DNA isolation object henceforth is called GAD-TAB1.

[0029] In order to determine the location in TAK1 which manages an interaction with example 2.TAB1, a series of LexA-TAK1 deletion chimera objects were examined by 2-hybrid measurement. Joint transformation of the expression vector which carries out the code of the overall length TAK1 united with the LexADNA-joint domain or its deletion structure was carried out to the yeast reporter stock L40 with pGAD-TAB1. In addition, DNA which carries out the code of each deletion structure of TAK1 produced the overall length TAK1 from DNA which carries out a code.

[0030] Moreover, said plasmid pGAD-TAB1 is obtained by carrying out subcloning of the TAB1cDNA to the EcoRI part of pBS (W. O.Bullock et al., Biotechniques Vol.5, p.376 (1987)). The interaction between the fusion proteins discovered by this plasmid is 40mM. It is shown by the capacity of the yeast stock increased on the plate of the SC-His culture medium containing 3-AT. This result is shown in drawing 1 . (+) with which TAK1 or its deletion object, and TAB1 interacted to this drawing Nakamigi side, and (-) which did not have an end again are shown. From this result, it was shown that TAB1 interacts with N-end side domain of TAK1.

[0031] The protein which interacts with example 3.TAK1 may include both an upstream control section and a label-lower stream of a river. If TAB1 plays a role to activation of TAK1, those coincidence manifestations will be expected to affect the activity of TAK1 in yeast. this invention persons are developing the system for measuring mammals MAPKKK activity in a yeast pheromone-induction MAPK path (265 270 K Yamaguchi et al., Science, Vol. p.2008 (1995); K Irie and others, Science, Vol. p.1716 (1994)). The gestalt (TAK1deltaN) by which TAK1 was activated is Ste11. It can substitute for MAPKKK activity.

[0032] That is, a pheromone-activation MAPK trajectory consists of Ste11, Ste7 and Fus3, or Kss1 kinase, and these correspond to MAPKKK, MAPKK, and MAPK, respectively. Such yeast protein kinases act one after another, and transmit a signal to a transcription factor Ste12. this Ste12 activates the imprint of the junction specific (mating specific) gene like FUS1 (I. -- Herskowitz, Cell, Vol.80, p.187 (1995); D.E.Levin et al. --) Curr.Opin.Cell Biol., Vol.7, and p.197 (1995); J.Schultz ** -- Jr.Curr.Opin.Gene Dev., No.5, and p.31 (1995).

[0033] FUS1p: :HIS3 reporter gene makes it possible to act as a monitor according to the capacity (His phenotype) of a cell to change including the FUS1 upstream activation array connected with HIS3 open reading frame, and to increase the signal activity in three shares of his3deltaFUS1p::HIS on a SC-His culture medium. his3deltaste11deltaFUS1p::HIS3STE7P368 (proline permutation in serine -368) stock is His. - It has phenotype (265 K Irie et al., Science, Vol. p.1716 (1994)).

[0034] The manifestation of TAK1deltaN in this stock is His+. Phenotype is brought about (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)). Therefore, the activation mold of TAK1 is Ste7P368. - It can substitute for Ste11 activity anaclitic. However, the manifestation of an overall length TAK1 does not recover ste11delta variation, but it is suggested that yeast does not have the activator of the assumption for TAK1 (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)).

[0035] GAD-TAB1 structure was examined using the yeast MAPK path about those capacity that complements ste11delta variation with the bottom of existence of TAK1. Namely, 1984-P shares (his3deltaste11deltaFUS1p::HIS3STE7P368) of yeast SY A transformation is carried out by pNV11-HU11(TAK1deltaN)+pGAD10(GAD) (Clontech);pNV11-HU11F(TAK1)+pGAD10;pNV11-HU11 F+pGAD-TAB1; or pNV11+pGAD-TAB1. And the transformant was wound on the SC-His plate, and it incubated at 30 degrees C.

[0036] In addition, the transformation of 1984-P shares of above SY is carried out to the basis of control of 1984 shares (his3deltaste11deltaFUS1p;;HIS3) of SY(s) of CYC1 promotor by plasmid pNC318-p368 containing STE7P368 (265 K Irie et al., Science, Vol. p.1716 (1994)). Moreover, above-mentioned plasmid pNV11-HU11 and above-mentioned pNV11-HU11F discover TAK1 of TAK1deltaN (amino acid 21-579) shortened by the basis of control of TDH3 promotor, and an overall length, respectively (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)).

[0037] A result is shown in drawing 2 . A left-hand side panel shows whether the coincidence

manifestation of whether the examined yeast stock discovered TAK1deltaN or TAK1 and GAD-TAB1 was carried out. A right-hand side panel shows growth of the cell on a SC-His plate. Each patch shows the result about the independent transformant. The joint transformation of GAD-TAB1 and TAK1 recovered Ste11 deficit. This result shows that TAB1 reinforces the function of TAK1.

[0038] In order to determine whether TAK1 activity increases in the yeast which discovers example 4.TAB1, TAK1-K63W (K -- Yamaguchi et al. --) by which the lysine of the 63rd place of TAK1 which supports a (Hemagglutinin HA)-origin C-end epitope, and a TAK1 variant [ATP-bonding site inactive in catalyst was permuted by the tryptophan The transformation of the manifestation DNA vector of Science, Vol.270, and p.2008 (1995)] was carried out to the yeast cell under the nonexistence of TAB1 gene, or existence.

[0039] The frame was doubled with the TAK1 coding sequence and C-end of TAK1-K63W, and the DNA array which carries out the code of the epitope recognized by monoclonal antibody 12CA5 to HA was connected by polymerase chain reaction (PCR). All structures are discovered from a TDH promotor. TAB1 manifestation plasmid pGAP-HTH9M discover 68 amino acid of C-end of TAB1. YEpGAP112 is multi-copy TRP1 plasmid containing TDH3 promotor [H.Banno et al., Mol.Cell Biol.13, and 475 (1993)].

[0040] It amplified by PCR using 3'-primer-5'-GGGTCGACTACGGTGC-3' (array number: 3) which contains a SalI part in 5'-primer 5'-GAGAATTCATGCGGCAAAGC-3' (array number: 2) which contains an ECORI part and an ATC codon for the coding sequence of 68 amino acid of C-end of TAB1, and a list. pGAD-HTH9M were produced by inserting in the ECORI-SalI gap of YEpGAP112 the ECORI-SalI fragment of 240bp(s) produced by PCR.

[0041] A result is shown in drawing 3 . pGAP-HTH9 which carries out a transformation by the plasmid which carries out the code of the plasmid which carries out the code of TAK1-HA of the above 1984 shares of yeast SY as mentioned above, or TAK1-K63W, and carries out the code of the empty vector YEpGAP112 (-) or TAB1 to this transformant further -- M (+) was introduced. Immunoprecipitation of TAK1-HA (-) or TAK1-K63 W-HA (KN) was carried out from each cell extract, and the immune precipitate was covered over in vitro kinase measurement. Specifically the 60ml yeast cell culture object was made to increase to the optical density 0.8 in 600nm, and the cell extract was prepared with the lysis buffer solution (265 K Irie et al., Science, Vol. p.1716 (1994)), and at-long-intervals alignment separation was carried out in 100,000g for 30 minutes.

[0042] Supernatant liquid was applied to immunoprecipitation with the antibody to HA. That is, one part (300microl) of supernatant liquid was mixed with the antibody of 2microl, and the protein A-sepharose of 90microl, the lysis buffer solution washed the immune complex 3 times, and business was carried out to kinase measurement (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)). The result of the immuno blot of each immune precipitate by monoclonal antibody 12CA5 to HA showed that TAK1-HA of the about same amount or TAK1-K63 W-HA were collected in each sample. Thereby, it was shown that the manifestation of TAB1 does not influence the amount of manifestations of TAK1.

[0043] TAK1 which carried out immunoprecipitation was measured according to the capacity which rearranges and activates XMEK2 (SEK1), and measured the activity of this recombination XMEK2 (SEK1) according to the capacity to phosphorize (KN) p38 [inactive in catalyst] (MPK2) (270 K Yamaguchi et al., Science, No. p.2008 (1995)). Autoradiography detected the phosphorylation of KH-p38 (MPK2) after electrophoresis. There was no extract in which kinase measured value is shown without an enzyme extract. This level is equivalent to the base activity of XMEK2. The experiment was conducted at least 3 times and the same result was obtained.

[0044] A result is shown in drawing 3 . The result of kinase measurement of TAK1-HA and TAK1-K36 W-TAK1 showed that TAB1 made the kinase activity of TAK1 increase. This increment in activity was not observed in the immune complex from the cell which discovers TAK1-K63WKN and TAB1, but it was shown that the observed kinase activity originates in TAK1. According to the place which these results show, TAB1 activates the kinase activity of TAK1 by coupling directly with the catalyst domain of TAK1.

[0045] In order to acquire the overall-length coding sequence of example 5.TAB1, the Homo sapiens

nephrocyte library was screened using as a probe the partial array of cDNA of TAB1 which was able to be obtained from the aforementioned yeast 2-hybrid system. Two independent clones are Kozak. cDNA of 3.1kbs containing one open reading frame (ORF) which begins from the initiation methionine codon which is in agreement with Cong Seth Sas was brought about. 5'-RACE-Ready The 5'-end was determined by the 5'RACE method using cDNA (Clonfech).

[0046] The nucleotide sequence (CCAAATGG) in N-end of an assumption of a coding sequence is Kozak. Consensus (M. Kozak, J.Cell Biol.Vol.108, and p.229 (1989)) Corresponding [and], an ATG codon does not exist before that. The nucleotide sequence of TAB1 was determined by the dideoxy nucleotide chain termination method. The amino acid sequence was presumed from the nucleotide sequence of overall-length TAB1cDNA. Consequently, two sorts, the clone whose 185th base is a cytosine, and the clone which is an adenine, were obtained. In the 185th, in the clone of a cytosine, the 52nd amino acid was carrying out [the 52nd amino acid / in / in the 185th / the clone of an adenine] the code of the arginine for the serine.

[0047] The nucleotide sequence of the clone whose 185th nucleotide is a cytosine is shown in the array number 1, and an amino acid sequence is shown in drawing 4 and the array number 1. Moreover, the nucleotide sequence of the clone whose 185th nucleotide is an adenine is shown in the array number 4, and an amino acid sequence is shown in the array number 4. In addition, subcloning of the cDNA of the clone of a cytosine was carried out to EcoRI and the SmaI part of pBS, the 185th nucleotide was produced as plasmid TABI-f -4, subcloning of the 185th nucleotide was carried out to the EcoRI part of pBS, and cDNA of the clone of an adenine was produced as plasmid pBS-TAB1.

[0048] The Escherichia coli containing plasmid pBS-TAB1 is Escherichiacoli. It is named HB [101 (pBS-TAB1)], and is FERM to National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology. It ****ed as BP-5508 on April 19, 1996. Moreover, the Escherichia coli containing plasmid TABI-f -4 is Escherichia. coli It is named DH5alpha (TABI-f -4), and is FERM to National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology. It ****ed as BP-5599 on July 19, 1996. About the experiment shown below, it carried out using the clone which has the nucleotide sequence shown in array number:1.

[0049] drawing 4 -- setting -- A -- Ala -- in b, E for Asp Glu [C] [Cys] F -- Phe -- G -- Gly -- in I, K for Ile Lys [H] [His] L -- Leu -- M -- Met -- N -- Asn -- P -- Pro -- Q -- Gln -- R -- Arg -- S -- Ser -- T -- in V, W shows Val and Trp and Y show Tyr for Thr, respectively. 68 amino acid of C-end in GAD-TAB1 isolated using the yeast 2-hybrid system is surrounded with the box.

[0050] The array of N-end of TAK1 is shown by arrangement to show the field which shows the bottom of similar to this segment of TAK1. the amino acid of TAK1 -- receiving -- the same amino acid and the amino acid saved -- an asterisk and a point show, respectively. 55kDa(s) which what kind of known protein does not have clear similarity from this ORF, and do not contain any motif in which a biochemical function is shown, either The protein of the 504 amino acid which has molecule size was expected.

[0051] TAB1 in the human cell of example 6. versatility Northern blot analysis analyzed the pattern of a manifestation of mRNA. The probe of the human tissue blot (Clontech) by mRNA prepared from the organization of 16 was carried out by 32P-labeling TAB1cDNA, and it applied to autoradiography. This result is shown in drawing 5 . Each lane contained mRNA of 2microg. a probe -- Multiprime labeling Kit (Amersham) -- using -- [alpha-32P]-dCTP -- an indicator -- carrying out -- and H.Shibuya ** -- Nature, Vol.357, and p.700 (1992) It is indicated and was made to make and hybridize. From all the organizations that examined, the main imprint object of about 3.5 kbs was detected.

[0052] example 7., in order to check that a meeting with TAB1 and TAK1 takes place in a mammals cell TAK1 (HA-TAK1) (K -- Yamaguchi et al. --) by which the indicator was carried out by HA epitope By the manifestation plasmid which produces TAB1 (Myc-TAB1) by which the indicator was carried out by the expression vector and Myc epitope which produce Science, Vol.270, and p.2008 (1995) Transience (transient) was transfected in MC3 T3-E1 rat osteoblast (S. Ohta et al., FEBS Lett., Vol.314, and p.356 (1992)). The latter plasmid was obtained as follows.

[0053] pCS2MT vector containing six copies of the Myc epitope (LEQKLISEEDLN) (1 character

representation of an amino acid sequence) recognized in overall-length TAB1cDNA by the monoclonal antibody nine E10 to Myc (D. L.Tumer et al., GenesDev., Vol.8, and p.1434 (1994)) Subcloning was carried out. In plasmid pCS2 MT-TAB1 obtained, a Myc epitope indicator doubles a frame and is connected with the DNA array corresponding to N-end of TAB1. pCSA2 MT-TAB1 was digested by BamHI and Xbal. The fragment was isolated and it inserted in the ECORI-Xbal part of the mammals expression vector pEF. This plasmid makes TAB1 discover from a Homo sapiens elongation factor 1alpha (EF1alpha) promotor.

[0054] the monoclonal antibody [as opposed to HA for a cell extract] 12 -- it applied to immunoprecipitation with the monoclonal antibody nine E10 (lane 3 of drawing 6) to CA5 (lane 2 of drawing 6), and Myc, or the contrast non-immunity IgG (lane 4 of drawing 6). The immune complex was washed, SDS-PAGE separated, you made it shift to a nitrocellulose, and the immuno blot was carried out using the antibody (upper lane of drawing 6) to Myc or the antibody (bottom lane of drawing 6) to HA.

[0055] The cell extract was directly covered over immuno blot analysis again (lane 1 of drawing 6). As shown in drawing 6 , Myc-TAB1 of most amount was detected in each immunoprecipitation, and it was shown that TAK1 may ********** with TAB1. By the mutual experiment which carries out the blot of the protein which carried out immunoprecipitation by the antibody to HA, the meeting with TAB1 and TAK1 was checked. These experiments show that TAB1 can meet with TAK1 in a mammals cell similarly also in yeast.

[0056] It considered whether the superfluous manifestation of example 8.TAB1 could activate the kinase activity of TAK1 in a mammals cell. MC3 T3-E1 cell was transfected transient by HA-TAK1 in (-) under (+) or nonexistence under existence of Myc-TAB1. a cell -- 20 ng(s)/ml TGF-beta 1 -- for 10 minutes -- processing -- (+) -- or as it does not process but indicated in the example 3 at (-) and a degree, immunoprecipitation of HA-TAK1 was carried out, and it measured about kinase activity. That is, the immuno blot of a part of immunoprecipitation was carried out by the antibody to HA. A result is shown in drawing 7 .

[0057] as the increment multiple over the amount of HA-TAK1 from the cell by which activity is not stimulated -- being shown -- an average of [from three experiments or more] -- it expressed as **SEM (graph of the drawing 7 upper case). HA-TAK1 did not phosphorize KH-p38 (MPK2) directly (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)). the middle -- a panel is an autoradiograph showing the phosphorylation of KN-p38 (MPK2). A lower-berth panel shows immuno blot analysis of each immunoprecipitation by monoclonal antibody 12CA5 to HA, and shows that TAK1-HA of the about same amount was collected in each sample. The data shown in the panel of the middle and the lower berth is a thing from a typical experiment.

[0058] According to the place which in vitro kinase measurement of TAK1 immune precipitate shows, TAK1 activity was stimulated in the cell in which even the bottom of the nonexistence of TGF-beta was transfected by TAB1. The activation of TAK1 by the superfluous manifestation of TAB1 was equal to the activation observed in the cell stimulated by TGF-beta which discovers only HA-TAK1.

[0059] Example 9. TGF-beta makes the amount of mRNA which carries out the code of the plasminogen activator inhibitor -1 (PAI-1) increase quickly (M. R.Keeton et al., J.Biol.Chem., Vol.266, and p.23048 (1991)). The reporter gene which contains the luciferase gene under a TGF-beta-inductivity pAI-1 gene promotor's control by the superfluous manifestation of the activation form (TAK1deltaN) of TAK1 is activated in configuration (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)). It examined whether the superfluous manifestation of TAB1 would bring about activation of a luciferase reporter gene.

[0060] A MvlLu cell Reporter plasmid p800neoLUC (M. Abe et al., Analyt.Biochem., Vol.216, and p.276 (1994)), the manifestation plasmid (K -- Yamaguchi et al. --) which carries out the code of TAB1 manifestation plasmid pEF-TAB1 or TAK1 Transient transfection was carried out using Science, Vol.270, and p.2008 (1995) by the calcium phosphate method (H. Shibuya et al., Nature, Vol.357, and p.700 (1992)). In addition, plasmid pEF-TAB1 contains the overall-length TAB1 coding sequence on the basis of control of an EF1alpha promotor, makes pEF cleave by ECORI, and is produced by inserting the ECORI fragment from plasmid TABI-f -4.

[0061] This plasmid TABI-f -4 is produced by carrying out subcloning of the TAB1cDNA to ECORI and the SmaI part of pBS. a cell — 30 ng(s)/ml Homo sapiens TGF-beta 1 — or it incubated for 20 hours without being accompanied by this, and the extract was prepared, and luciferase was measured (H. Shibuya et al., Mol.Cell Biol., Vol.14, and p.5812 (1994)). Luciferase activity was amended based on the manifestation of the beta-galactosidase.
[0062] That is, in order to amend transfection effectiveness, in all luciferase reporter experiments, coincidence transfection of the pXeX-beta-Gal vector (A. D.Johnson et al., Gene, Vol.147, and p.223 (1994)) was carried out. Measurement of the beta-galactosidase was performed according to a manufacturer's (Clontech) instructions using the cell melt prepared for luciferase measurement. Luciferase activity was shown as an increment multiple over the activity of the non-stimulating cell transfected by the vector. All transfection and luciferase measurement were performed at least 5 times, and each experiment was conducted 3 reams.
[0063] A result is shown in drawing 8 . KN shows TAK1-K63W [inactive in catalyst] among drawing. Data shows average**SEM of the luciferase activity from the experiment of 3 reams in a typical experiment. Although the superfluous manifestation of TAB1 guided the manifestation of a reporter gene also under the nonexistence of TGF-beta with TAK1, the superfluous manifestation of only TAK1 or TAB1 hardly affected the configuration-amount of luciferase activity. These researches show that TAB1 reinforces the activity of TAK1 in a mammals cell.
[0064] Although the superfluous manifestation of the variant of TAK1-K63W checked the luciferase activity stimulated by TGF-beta (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)), this will be for blocking the essential element in a path (sequestering). On the other hand, the superfluous manifestation of TAB1 reduces the inhibition effectiveness of TAK1-K63W, and suggests possibility of being absorbed by the superfluous manifestation whose TAB1 is TAK1-K63W.
[0065] 68 amino acid [TAB1 (437-504)] of C-end of example 10.TAB1 is enough in order to combine with TAK1 and to activate it, and it was suggested that N-end domain of TAB1 plays the control-role in the function of TAB1. In order to examine this possibility, the contracted form [TAB1 (1-418)] of TAB1 lacking in a C-end TAK1 joint domain was produced. About a MvlLu cell, it is p800nedUC. Transience was transfected with the reporter and the amount which shows TAB1 (1-418) or TAB1 (overall length) to drawing 9 of the expression vector which carries out a code, and they were complemented by the pEF control vector.
[0066] In addition, the expression vector which carries out a code produced TAB1 (1-418) as follows. Subcloning of the ECORI-HincII fragment (N-distal region of the amino acid 1-418 of TAB1 is contained) of 1.3kbs of plasmid TABI-f -4 was carried out to pKT10 vector, and pKT10-TAB1 (1-418) was produced. pEF was made to cleave by ECORI and SalI, and pEF-TAB1 (1-418) was produced by inserting the ECORI-SalI fragment from pKS10-TAB1 (1-418).
[0067] next, a cell — 30 ng(s)/ml TGF-beta 1 — or it incubated for 20 hours without being accompanied by this, and the cell melt was measured about luciferase activity. The numeric value was shown as % of the multiple of induction to the reference cell transfected by pEF. the luciferase by TGF-beta — being unguided (induction multiple 1) — it corresponds to 0%. Measurement of all transfection and luciferases was performed at least 3 times, and each experiment was conducted 3 reams. Average**SEM of the luciferase activity from the experiment of 3 reams in a typical experiment shows data.
[0068] A result is shown in drawing 9 . The superfluous manifestation of TAB1 (1-418) in a MvlLu cell controlled the activity of the reporter gene guided by TGF-beta stimulus. Therefore, TAB1 (1-418) acts as dominant-negative inhibitor of the gene expression guided by TGF-beta. These results show that TAB1 participates in signal transfer of TGF-beta.
[0069] It is possible that TAB1 combined with TAK1 guides a conformation change required for activation as a mechanism to which TAB1 guides activation of TAK1. Since removal of 20 amino acid in N-end of TAK1 brings about configuration-activation of protein kinase, N-end domain restricting a catalyst domain and checking kinase activity is suggested (270 K Yamaguchi et al., Science, Vol. p.2008 (1995)). TAB1 may cancel this negative control domain of TAK1 of that catalyst domain. C-end of TAB1 which functions as TAK1 binding site contains the serine and threonine rich field as well as the field found out by N-end of TAK1. Therefore, TAB1 is TGF-beta and TAK1. They are the

important signal transfer intermediate field between MAPKKK(s).
[0070]
[Layout Table]
array number: — die-length [of one array]: — mold [of 1560 arrays]: — number [of nucleic-acid chains]: — double strand topology: — class [of straight chain-like array]: — cDNA Array
GAATTCGTGG CCCGCAGGGT TCCTCCAAG ATG GCG GCG CAG AGG AGG AGC TTG 53 Met Ala Ala Gln Arg Arg Ser Leu 5 CTG CAG AGT GAG CAG CAG CCA AGC TGG ACA GAT GAC CTG CCT CTC TGC 101 Leu Gln Ser Glu Gln Gln Pro Ser Trp Thr Asp Asp Leu Pro Leu Cys 10 15 20 CAC CTC TCT GGG GTT GGC TCA GCC TCC AAC CGC AGC TAC TCT GCT GAT149 His Leu Ser GlyVal Gly Ser Ala Ser Asn Arg Ser Tyr Ser Ala Asp 25 30 35 40 GGC AAG GGC ACTGAG AGC CAC CCG CCA GAG GAC AGC TGG CTC AAG TTC 197 Gly Lys Gly Thr Glu Ser His Pro Pro Glu Asp Ser Trp Leu Lys Phe 45 50 55 AGG AGT GAG AACAAC TGC TTC CTG TAT GGG GTC TTC AAC GGC TAT GAT 245 Arg Ser Glu Asn Asn Cys Phe Leu Tyr Gly Val Phe Asn Gly Tyr Asp 60 65 70GGC AAC CGA GTG ACC AAC TTC GTG GCC CAG CGG CTG TCC GCA GAG CTC 293 Gly Asn Arg Val Thr Asn Phe Val Ala Gln Arg Leu Ser Ala Glu Leu 75 80 85 [0071]
CTG-CTG-GGC-CAG-CTG AAT-GCC-GAG-CAC-GCC GAG GCC GAT GTG CGG CGT 341 Leu Leu Gly Gln Leu Asn Ala-Glu-His-Ala-Glu-Ala-Asp-Val-Arg-Arg 90 95 100 GTG CTG CTG CAG GCC-TTC-GAT-GTG-GTG GAG AGG AGC TTC CTG GAG TCC 389 Val Leu Leu Gln Ala Phe Asp Val Val Glu Arg Ser Phe Leu Glu Ser 105 110 115 120 ATT GAC GAC GCC TTG GCT GAG AAG GCA AGC CTC CAG TCG CAA TTG CCA437 Ile Asp Asp AlaLeu Ala Glu Lys Ala Ser Leu Gln Ser Gln Leu Pro 125 130 135 GAG GGA GTCCCTCAG CAC CAG CTG CCT CCT CAG TAT CAG AAG ATC CTT 485 Glu Gly Val Pro Gln His Gln Leu Pro Pro Gln Tyr Gln Lys Ile Leu 140 145 150 GAG AGA CTC AAG ACG TTA GAG AGG GAA ATT TCG GGA GGGGCC ATG GCC 533 Glu Arg Leu Lys Thr Leu Glu Arg Glu Ile Ser Gly Gly Ala Met Ala 155 160 165 GTT GTG GCG GTCCTTCTC AAC AAC AAG CTC TAC GTC GCC AAT GTC GGT 581 Val Val Ala Val Leu Leu Asn Asn Lys Leu Tyr Val Ala Asn Val Gly 170 175 180 ACA AAC CGTGCACTT TTA TGC AAA TCG ACA GTG GAT GGG TTG CAG GTG 629 Thr Asn Arg Ala Leu Leu Cys Lys Ser Thr Val Asp Gly Leu Gln Val 185 190 195 200 ACA CAG CTG AAC GTG GAC CAC ACC ACA GAG AAC GAG GAT GAG CTC TTC 677 Thr Gln Leu Asn Val Asp His Thr Thr Glu Asn Glu Asp Glu Leu Phe 205 210 215 CGT CTT TCG CAGCTG GGC TTG GAT GCT GGA AAG ATC AAG CAG GTG GGG 725 Arg Leu Ser Gln Leu Gly Leu Asp Ala Gly Lys Ile Lys Gln Val Gly 220 225 230 [0072]
ATC-ATC-TGT-GGG-CAG GAG-AGC-ACC-CGG-CGG ATC GGG GAT TAC AAG GTT 773 Ile Ile Cys Gly Gln Glu Ser-Thr-Arg-Arg-Ile-Gly-Asp-Tyr-Lys-Val 235 240 245 AAA TAT GGC TAC ACG GAC ATT GAC CTT CTC AGC GCT GCC AAG-TCCAAA 821 Lys Tyr Gly Tyr Thr Asp Ile Asp Leu Leu Ser Ala Ala Lys Ser Lys 250 255 260 CCA ATCATC GCA GAGCCAGAA ATC CAT GGG GCA CAG CCG CTG GAT GGG 869 Pro Ile Ile Ala Glu Pro Glu Ile His Gly Ala Gln Pro Leu Asp Gly 265 270 275 280GTG ACGGGCTTC TTG GTG CTG ATG TCG GAG GGG TTG TAC AAG GCC CTA 917 Val Thr Gly Phe Leu Val Leu Met Ser Glu Gly Leu Tyr Lys Ala Leu 285 290 295 GAG GCA GCCCATGGG CCT GGG CAG GCC AAC CAG GAG ATT GCT GCG ATG 965 Glu Ala Ala His Gly Pro Gly Gln Ala Asn Gln Glu Ile Ala Ala Met 300 305 310 ATT GAC ACT GAG TTT GCC AAG CAG ACC TCC CTG GAC GCA GTG GCC CAG 1013 Ile Asp Thr Glu Phe Ala Lys Gln Thr Ser Leu Asp Ala Val Ala Gln 315 320 325 GCC GTC GTG GACCGGGTG AAG CGC ATC CAC AGC GAC ACC TTC GCC AGT 1061 Ala Val Val Asp Arg Val Lys Arg Ile His Ser Asp Thr Phe Ala Ser 330 335 340 GGT GGG GAG CGT GCC AGGTTC TGC CCC CGG CAC GAG GAC ATG ACC CTG 1109 Gly Gly Glu Arg Ala Arg Phe Cys Pro Arg His Glu Asp Met Thr Leu 345 350 355 360 CTA GTG AGG AAC TTT GGC TAC CCG CTG GGC GAA ATG AGC CAG CCC ACA 1157 Leu Val Arg Asn Phe Gly Tyr Pro Leu Gly Glu Met Ser Gln Pro Thr 365 370 375 CCG AGC CCAGCCCCA GCT GCA GGA GGA CGA GTG TAC CCT GTG TCT GTG 1205 Pro Ser Pro Ala Pro Ala Ala Gly Gly Arg Val Tyr Pro Val Ser Val 380 385 390 CCA TAC TCC AGC GCCCAGAGC ACC AGC AAG ACC AGC GTG ACCCTC TCC 1253 Pro Tyr Ser Ser Ala Gln Ser Thr Ser Lys Thr Ser Val Thr Leu Ser 395 400 405 CTTGTC ATG CCC TCCCAGGGC CAG ATG GTC AAC GGG GCT CAC AGT GCT 1301 Leu Val Met Pro-Ser-Gln-Gly-Gln Met Val Asn Gly Ala-His-Ser-Ala 410 415 420 TCC ACC CTG GAC GAA-GCC-ACC-CCC-ACC CTC-ACC-AAC-CAA-AGC CCG ACC 1349 Ser Thr-Leu-Asp-Glu-Ala

Thr Pro Thr Leu Thr-Asn-Gln-Ser-Pro-Thr 425 430 435 440 TTA ACC CTG CAGTCC ACC AAC ACG CAC ACG CAG AGC AGC AGC TCC AGC 1397 Leu Thr Leu Gln Ser Thr Asn Thr His Thr Gln Ser Ser Ser Ser Ser 445 450 455 TCT GAC GGA GGC CTC TTC CGC TCC CGG CCC GCC CAC TCG CTC CCG CCT 1445 Ser Asp Gly Gly Leu Phe Arg Ser Arg Pro Ala His Ser Leu Pro Pro 460 465 470 GGC GAG GAC GGT CGT GTT GAG CCC TAT GTG GAC TTT GCT GAG TTT TAC 1493 Gly Glu Asp Gly Arg Val Glu Pro Tyr Val Asp Phe Ala Glu Phe Tyr 475 480 485 CGC CTC TGG AGC GTG GAC CAT GGC GAG CAG AGC GTG GTG ACA GCA CCG 1541 Arg Leu Trp Ser Val Asp His Gly Glu Gln Ser Val Val Thr Ala Pro 490 495 500 TAGGGCAGCC GGAGGAATG 1560 [0073] array number: — die-length [of two arrays]: — mold [of 20 arrays]: — number [of nucleic-acid chains]: — single strand topology: — class [of straight chain-like array]: — synthetic DNA Array GAGAATTCAT GCGGCAAAGC 20 [0074] array number: — die-length [of three arrays]: — mold [of 16 arrays]: — number [of nucleic-acid chains]: — single strand topology: — class [of straight chain-like array]: — synthetic DNA Array GGGTCGACTA CGGTGC 16 [0075] die-length [of array number 4 array]: — mold [of 1560 arrays]: — number [of nucleic-acid chains]: — double strand topology: — class [of straight chain-like array]: — cDNA array GAATTCGTGG CCCGCAGGGT TCCTCCAAG ATG GCG GCG CAG AGG AGG AGC TTG 53 Met Ala Ala Gln Arg Arg Ser Leu 5 CTGCAG AGT GAG CAG CAG CCA AGC TGG ACA GAT GAC CTG CCT CTC TGC 101 Leu Gln Ser Glu Gln Gln Pro Ser Trp Thr Asp Asp Leu Pro Leu Cys 10 15 20 CAC CTC TCT GGG GTT GGC TCA GCC TCC AAC CGC AGC TAC TCT GCT GAT 149 His Leu Ser Gly Val Gly Ser Ala Ser Asn Arg Ser Tyr Ser Ala Asp 25 30 35 40 GGC AAGGGC ACT GAG AGCCAC CCG CCA GAG GAC AGA TGG CTC AAG TTC 197 Gly Lys Gly Thr Glu Ser His Pro Pro Glu Asp Arg Trp Leu Lys Phe 45 50 55 AGG AGT GAG AACAAC TGC TTC CTG TAT GGG GTC TTC AAC GGC TAT GAT 245 Arg Ser Glu Asn Asn Cys Phe Leu Tyr Gly Val Phe Asn Gly Tyr Asp 60 65 70 GGC AAC CGA GTG ACC AAC TTC GTG GCC CAG CGG CTG TCC GCA GAG CTC 293 Gly Asn Arg Val Thr Asn Phe Val Ala Gln Arg Leu Ser Ala Glu Leu 75 80 85 [0076] CTG-CTG-GGC-CAG-CTG AAT-GCC-GAG-CAC-GCC GAG GCC GAT GTG CGG CGT 341 Leu Leu Gly Gln Leu Asn Ala-Glu-His-Ala-Glu-Ala-Asp-Val-Arg-Arg 90 95 100 GTG CTG CTG CAG GCC TTC GAT GTG GTG GAG AGG AGC TTC CTG GAG TCC389 Val Leu Leu GlnAla Phe Asp Val Val Glu Arg Ser Phe Leu Glu Ser 105 110 115 120ATT GAC GAC GCC TTG GCT GAG AAG GCA AGC CTC CAGTCG CAA TTG CCA 437 Ile Asp Asp Ala Leu Ala Glu Lys Ala Ser Leu Gln Ser Gln Leu Pro 125 130 135 GAG GGA GTCCCTCAG CAC CAG CTG CCT CCT CAG TAT CAG AAG ATC CTT 485 Glu Gly Val Pro Gln His Gln Leu Pro Pro Gln Tyr Gln Lys Ile Leu 140 145 150 GAG AGA CTC AAG ACG TTA GAG AGG GAA ATT TCG GGA GGGGCC ATG GCC 533 Glu Arg Leu Lys Thr Leu Glu Arg Glu Ile Ser Gly Gly Ala Met Ala 155 160 165 GTT GTG GCG GTCCTTCTC AAC AAC AAG CTC TAC GTC GCC AAT GTC GGT 581 Val Val Ala Val Leu Leu Asn Asn Lys Leu Tyr Val Ala Asn Val Gly 170 175 180 ACA AAC CGTGCACTT TTA TGC AAA TCG ACA GTG GAT GGG TTG CAG GTG 629 Thr Asn Arg Ala Leu Leu Cys Lys Ser Thr Val Asp Gly Leu Gln Val 185 190 195 200 ACA CAG CTG AAC GTG GAC CAC ACC ACA GAG AAC GAG GAT GAG CTC TTC 677 Thr Gln Leu Asn Val Asp His Thr Thr Glu Asn Glu Asp Glu Leu Phe 205 210 215 CGT CTT TCG CAGCTG GGC TTG GAT GCT GGA AAG ATC AAG CAG GTG GGG 725 Arg Leu Ser Gln Leu Gly Leu Asp Ala Gly Lys Ile Lys Gln Val Gly 220 225 230 [0077] ATC-ATC-TGT-GGG-CAG GAG-AGC-ACC-CGG-CGG ATC GGG GAT TAC AAG GTT 773 Ile Ile Cys Gly Gln Glu Ser-Thr-Arg-Arg-Ile-Gly-Asp-Tyr-Lys-Val 235 240 245 AAA TAT GGC TAC ACG GAC ATT GAC CTT CTC AGC GCT GCC AAG-TCCAAA 821 Lys Tyr Gly Tyr Thr Asp Ile Asp Leu Leu Ser Ala Ala Lys Ser Lys 250 255 260 CCA ATCATC GCA GAGCCAGAA ATC CAT GGG GCA CAG CCG CTG GAT GGG 869 Pro Ile Ile Ala Glu Pro Glu Ile His Gly Ala Gln Pro Leu Asp Gly 265 270 275 280GTG ACGGGCTTC TTG GTG CTG ATG TCG GAG GGG TTG TAC AAG GCC CTA 917 Val Thr Gly Phe Leu Val Leu Met Ser Glu Gly Leu Tyr Lys Ala Leu 285 290 295 GAG GCA GCC CAT GGG CCTGGGCAG GCC AAC CAG GAG ATT GCT GCG ATG 965 Glu Ala Ala His Gly Pro Gly Gln Ala Asn Gln Glu Ile Ala Ala Met 300 305 310 ATTGAC ACT GAG TTT GCCAAGCAG ACC TCC CTG GAC GCA GTG GCC CAG 1013 Ile Asp Thr Glu Phe Ala Lys Gln Thr Ser Leu Asp Ala Val Ala Gln 315 320 325 GCC GTC GTG GACCGGGTG AAG CGC ATC CAC AGC GAC ACC TTC GCC AGT 1061 Ala Val Val Asp Arg Val Lys Arg Ile His Ser Asp Thr Phe Ala Ser 330 335 340 GGT GGG

GAG CGT GCC AGGTTC TGC CCC CGG CAC GAG GAC ATG ACC CTG 1109 Gly Gly Glu Arg Ala Arg Phe Cys Pro Arg His Glu Asp Met Thr Leu 345 350 355 360 CTA GTG AGG AAC TTT GGC TAC CCG CTG GGC GAA ATG AGC CAG CCC ACA 1157 Leu Val Arg Asn Phe Gly Tyr Pro Leu Gly Glu Met Ser Gln Pro Thr 365 370 375 CCG AGC CCAGCCCCA GCT GCA GGA GGA CGA GTG TAC CCT GTG TCT GTG 1205 Pro Ser Pro Ala Pro Ala Ala Gly Gly Arg Val Tyr Pro Val Ser Val 380 385 390 CCA TAC TCC AGC GCCCAGAGC ACC AGC AAG ACC AGC GTG ACCCTC TCC 1253 Pro Tyr Ser Ser Ala Gln Ser Thr Ser Lys Thr Ser Val Thr Leu Ser 395 400 405 CTTGTC ATG CCC TCCCAGGGC CAG ATG GTC AAC GGG GCT CAC AGT GCT 1301 Leu Val MetPro Ser Gln Gly Gln Met Val Asn Gly Ala His Ser Ala 410 415 420 TCC ACC CTG GAC GAA-GCC-ACC-CCC-ACC CTC-ACC-AAC-CAA-AGC CCG ACC 1349 Ser Thr-Leu-Asp-Glu-Ala Thr Pro Thr Leu Thr-Asn-Gln-Ser-Pro-Thr 425 430 435 440 TTA ACC CTG CAG TCC ACC AAC ACG CAC ACG CAG AGC AGC AGC TCC AGC1397 Leu Thr Leu Gln Ser Thr Asn Thr His Thr Gln Ser Ser Ser Ser Ser 445 450 455 TCT GAC GGAGGCCTC TTC CGC TCC CGG CCC GCC CAC TCG CTC CCG CCT 1445 Ser Asp Gly Gly Leu Phe Arg Ser Arg Pro Ala His Ser Leu Pro Pro 460 465 470 GGC GAG GAC GGT CGTGTTGAG CCC TAT GTG GAC TTT GCT GAG TTT TAC 1493 Gly Glu Asp Gly Arg Val Glu Pro Tyr Val Asp Phe Ala Glu Phe Tyr 475 480 485 CGCCTC TGG AGC GTGGACCAT GGC GAG CAG AGC GTG GTG ACA GCA CCG 1541 Arg Leu Trp Ser Val Asp His Gly Glu Gln Ser Val Val Thr Ala Pro 490 495 500 TAGGGCAGCC GGAGGAATG1560

[Translation done.]